PUIZUSUSZOSEZ BIUZEUUM DT15 Rec'd PCT/PTO 2 7 DEC 2004

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We claim:

1. A variant humanized CC49 antibody, comprising:

a light chain complementarity determining region (L-CDR)1, a L-CDR2, and a L-CDR3, a heavy chain complementarity determining region (H-CDR)1, a H-CDR2, and a H-CDR3,

wherein a L-CDR3 of the variant humanized CC49 antibody or of a functional fragment of the variant humanized CC49 antibody comprises a non-conservative amino acid substitution, and wherein the variant humanized CC49 antibody has a high binding affinity for TAG-72, compared to a parent CC49 antibody.

- 2. The variant antibody of claim 1, wherein the non-conservative substitution is a tyrosine to proline substitution.
- 3. The variant antibody of claim 1, wherein the non-conservative substitution is at position 91.
- 4. The variant antibody of claim 1, wherein the non-conservative substitution is at a position that corresponds to a ligand contact residue.
- 5. The variant antibody of claim 1, wherein the functional fragment is an Fab fragment, an Fv fragment, or an F(ab')₂ fragment.
- 6. The variant antibody of claim 1, wherein the L-CDR1 and L-CDR2 are a human antibody L-CDR1 and L-CDR2, respectively.
- 7. The variant antibody of claim 1, wherein the L-CDR3, H-CDR1, H-CDR2, and H-CDR3 are from a murine CC49 antibody.
- 8. The variant antibody of claim 1, wherein the high binding affinity is at least about $1.2 \times 10^{-8} M$.

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- 9. The variant antibody of claim 8, wherein the high binding affinity is at least about 1.5×10^{-8} , about 2.0×10^{-8} , about 2.5×10^{-8} , about 3.0×10^{-8} , about 3.5×10^{-8} , about 4.0×10^{-8} , about 4.5×10^{-8} , or about 5.0×10^{-8} M.
- 10. The variant antibody of claim 1, wherein the antibody is minimally immunogenic.
- 11. The variant antibody of claim 1, wherein the antibody further comprises an effector molecule.
- 12. The variant antibody of claim 11, wherein the effector molecule is a detectable label.
- 13. The variant antibody of claim 12, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.
- 14. The variant antibody of claim 11, wherein the effector molecule is a toxin.
- 15. The variant antibody of claim 14, wherein the toxin is a chemotherapeutic drug, a radioactive isotope, a bacterial toxin, a viral toxin, a cytokine or a venom protein.
- 16. The variant antibody of claim 1, further comprising at least one additional non-conservative amino acid substitution in the L-CDR1.
- 17. The variant antibody of claim 1, further comprising at least one additional non-conservative amino acid substitution in the L-CDR2, or L-CDR3.
- 18. The variant antibody of claim 1, further comprising at least one non-conservative amino acid substitution in the H-CDR1.

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- 19. The antibody of claim 1, further comprising at least one non-conservative amino acid substitution in the H-CDR2, or H-CDR3.
- 20. A humanized CC49 antibody, wherein a nucleic acid sequence encoding the antibody has an ATCC Accession number comprising ATCC Accession number PTA-4182 or ATCC Accession number PTA-4183.
- 21. A nucleic acid molecule encoding the variant humanized monoclonal antibody of claim 1.
- 22. A vector comprising the nucleic acid of claim 21.
- 23. A variant humanized CC49 antibody, comprising:

a variable light framework region and a variable heavy framework region of a human antibody;

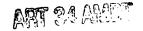
a light chain complementarity determining region (L-CDR)1, a L-CDR2, a L-CDR3, a heavy chain complementarity determining region (H-CDR)1, a H-CDR2, and a H-CDR3, wherein at least one complementarity determining region (CDR) is a human antibody CDR and remaining CDRs are murine CC49 antibody CDRs;

a non-conservative substitution of a first residue, wherein the first residue is in the L-CDR3 of the variant antibody; and

a substitution of a second residue, wherein the second residue is in a any L-CDR or H-CDR of the variant antibody;

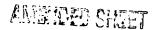
wherein the humanized CC49 antibody has a high binding affinity for TAG-72 and is minimally immunogenic, compared to a parent CC49 antibody.

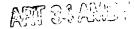
24. The variant antibody of claim 23, wherein the non-conservative substitution of the first residue is a tyrosine to proline substitution.



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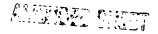
- 25. The variant antibody of claim 23, wherein the non-conservative substitution of the first residue is at position 91.
- 26. The variant antibody of claim 25, wherein the non-conservative substitution of the first residue at position 91 is a tyrosine to proline substitution.
- 27. The variant antibody of claim 23, wherein the antibody further comprises an effector molecule.
- 28. The variant antibody of claim 27, wherein the effector molecule is a detectable label.
- 29. The variant antibody of claim 28, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.
- 30. The variant antibody of claim 27, wherein the effector molecule is a toxin.
- 31. The variant antibody of claim 30, wherein the toxin is a chemotherapeutic drug, a radioactive isotope, a bacterial toxin, a viral toxin, a cytokine or a venom protein.
- 32. A method of detecting a TAG-72-expressing tumor in a subject, comprising:
 contacting a sample obtained from the subject with the variant
 antibody of claim 1 for a sufficient amount of time to form an immune complex; and
 detecting the presence of the immune complex, wherein the presence
 of the immune complex demonstrates the presence of the TAG-72-expressing tumor.
- 33. The method of claim 32, wherein the tumor is a colorectal tumor, a gastric tumor, a pancreatic tumor, a breast tumor, a lung tumor, an adenocarcinoma, or an ovarian tumor.





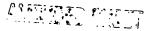
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- 34. The method of claim 32, wherein the variant antibody further comprises an effector molecule.
- 35. The method of claim 34, wherein the effector molecule is a detectable label.
- 36. The method of claim 35, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.
- 37. The method of claim 32, further comprising contacting the variant antibody with a secondary antibody.
- 38. The method of claim 37, wherein the secondary antibody further comprises a detectable label.
- 39. A method of detecting a TAG-72-expressing tumor in a subject, comprising:
 administering the variant antibody of claim 1 to the subject for a
 sufficient amount of time to form an immune complex; and
 detecting the presence of the immune complex, wherein the presence
 of the immune complex demonstrates the presence of the TAG-72-expressing tumor.
- 40. The method of claim 39, wherein the variant antibody further comprises an effector molecule.
- 41. The method of claim 40, wherein the effector molecule is a detectable label.
- 42. The method of claim 41, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.



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- 43. The method of claim 39, wherein the tumor is a colorectal tumor, a gastric tumor, a pancreatic tumor, a breast tumor, a lung tumor, an adenocarcinoma, or an ovarian tumor.
- 44. A method of treating a subject having a tumor that expresses TAG-72, comprising administering to the subject a therapeutically effective amount of the variant antibody of claim 1, wherein administering the therapeutically effective amount of the variant antibody of claim 1 inhibits the growth of the tumor or reduces the size of the tumor, thereby treating the subject.
- 45. The method of claim 44, wherein the administration of a therapeutically effective amount of the variant antibody of claim 1 does not elicit a human antimurine antibody response in a subject.
- 46. The method of claim 44, wherein the tumor is a colorectal tumor, a gastric tumor, a pancreatic tumor, a breast tumor, a lung tumor, an adenocarcinoma, or an ovarian tumor.
- 47. The method of claim 44, wherein the variant antibody further comprises an effector molecule.
- 48. The method of claim 47, wherein the effector molecule is a toxin.
- 49. The method of claim 48, wherein the toxin is a chemotherapeutic drug, a radioactive isotope, a bacterial toxin, a viral toxin, a cytokine, or a venom protein.
- 50. The method of claim 49, wherein the variant antibody comprising a radioactive isotope is used in radioimmunotherapy.
- 51. A method of treating a subject having a tumor that expresses TAG-72, comprising:



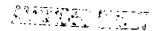
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administering the variant antibody of claim 1 to the subject for a sufficient amount of time to form an immune complex, wherein the variant antibody comprises a radioactive isotope;

detecting the presence of the immune complex with a hand-held gamma counter, wherein the presence of the immune complex demonstrates the presence of the TAG-72-expressing tumor; and

removing the tumor surgically, thereby treating the subject.

- 52. A pharmaceutical composition comprising a therapeutically effective amount of the variant antibody of claim 1 in a pharmaceutically acceptable carrier.
- 53. A kit, comprising a container comprising the variant antibody of claim 1.
- 54. The kit of claim 53, further comprising a container containing an antigen, a container containing a secondary antibody conjugated to a chemical compound, instructions for using the kit, or any combination thereof.
- 55. The variant antibody of claim 1, wherein the L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3 are the parent CC49 antibody L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3, respectively.
- 56. The variant antibody of claim 1, wherein the parent humanized CC49 antibody is HuCC49V10.
- 57. The variant antibody of claim 1, wherein the non-conservative substitution is a tyrosine to proline substitution at position 91.
- 58. The variant antibody of claim 57, further comprising a substitution of a second residue, wherein the second residue is in the L-CDR1, L-CDR2, or L-CDR3.
- 59. The variant antibody of claim 58, wherein the substitution of the second residue is in L-CDR1.



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- 60. The variant antibody of claim 59, wherein the substitution of the second residue is at position 27b of the L-CDR1.
- 61. The variant antibody of claim 60, wherein the substitution of the second residue is a valine to leucine substitution.
- 62. The variant antibody of claim 23, wherein the parent CC49 antibody is HuCC49V10.
- 63. The variant antibody of claim 23, wherein the substitution of the second residue is in the L-CDR1, L-CDR2, or L-CDR3 of the variant antibody.
- 64. The variant antibody of claim 63, wherein the substitution of the second residue is in L-CDR1.
- 65. The variant antibody of claim 64, wherein the substitution of the second residue is at position 27b of the L-CDR1.
- 66. The variant antibody of claim 65, wherein the substitution of the second residue is a valine to leucine substitution.
- 67. The variant antibody of claim 23, wherein the non-conservative substitution of the first residue at position 91 is a tyrosine to proline substitution, the substitution of the second residue at position 27b is a valine to leucine substitution, the L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3 are the parent CC49 antibody L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3, respectively, and the parent CC49 antibody is HuCC49V10.

